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Age and growth of the dusky grouper *Epinephelus marginatus* (Lowe 1834) in an exploited population of the western Mediterranean Sea

O. Reñones*†, C. Piñeiro‡, X. Mas* and R. Goñi*

*Centro Oceanográfico de Baleares, Instituto Español de Oceanografía, Apdo. 291, 07080 Palma de Mallorca, Spain and ‡Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, 36280 Vigo, Spain

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The age and growth of the dusky grouper, *Epinephelus marginatus*, in the Balearic Islands (western Mediterranean) were studied by otolith analysis from a sample of 358 specimens ranging in total length (L_T) from 6·6 to 105·6 cm. The specimens came from commercial artisanal and recreational spear fisheries between 1999 and 2003. Otoliths grew asymmetrically throughout the range of L_T studied, showing a clear pattern of alternating translucent and opaque bands. Marginal increment analysis of specimens up to 8 years-old indicated that a single opaque band was formed each year during spring and summer. Whole otoliths allowed ageing specimens up to 10 years old, but above that age whole otoliths yielded lower age estimates than sectioned otoliths. The maximum estimated age was 61 years, which significantly extends the estimated life span of the species from a maximum of 36 years in a previous study. The von Bertalanffy growth parameters were estimated as $L_\infty = 95{\cdot}5$ cm L_T, $K = 0{\cdot}087$ and $T_O = -1{\cdot}12$. The study revealed differences in mean L_T at age and age structure between the shallow- and deep-water samples which may be attributed to different fishing pressure and environmental conditions.

Key words: age and growth; *Epinephelus marginatus*; otolith sections; western Mediterranean.

INTRODUCTION

The dusky grouper *Epinephelus marginatus* (Lowe 1834) (Pisces: Serranidae, subfamily Epinephelinae) occurs from the British Isles to South Africa and from Bermuda to Argentina, and is widespread in the Mediterranean Sea but not the Black Sea (Heemstra & Randall, 1993; Rico & Acha, 2003; Irigoyen *et al.*, 2005). This species inhabits shelter-rich, hard substrata on the continental shelf, reaching maximum densities beyond 50 m depth. Because of its large size, fighting ability, bathymetric distribution and behaviour, *E. marginatus* is considered the most prized species for the recreational spear fishery and an emblematic species in the Mediterranean Sea.

†Author to whom correspondence should be addressed. Tel.: +34 971 401877; fax: +34 971 404945; email: olga.renones@ba.ieo.es

Groupers are long-lived, slow-growing fishes with low rates of natural mortality (Manooch, 1987). These characteristics combined with their reproductive pattern (most of them protogynous hermaphrodites) and behaviour makes them highly susceptible to overexploitation (Bannerot *et al.*, 1987; Bullock *et al.*, 1992; Sadovy, 1993; Huntsman & Schaaf, 1994; Armsworth, 2001). In the Mediterranean Sea the high spear fishing pressure exerted on *E. marginatus* populations in shallow waters, together with the anthropogenic perturbation of coastal habitats and the growing accessibility of the deeper segments of the populations to the longline fisheries, have seriously depleted its populations.

Epinephelus marginatus is the most common grouper species in the Balearic Archipelago (western Mediterranean Sea) (Fig. 1). As in other Mediterranean regions its populations have been depleted (Coll *et al.*, 1999, 2004; Reñones *et al.*, 1999) and currently the species is a valuable by-catch of the commercial fishery, while it remains the most sought after species of the recreational spear fishery. The commercial and recreational fisheries are seasonal, with most catches occurring during summer and autumn in relation with the maximum trophic and reproductive activity of the species. Fisheries are regulated by a minimum landing size (MLS) of 45 cm total length (L_T).

Knowledge of the growth pattern of exploited species is fundamental to establishing MLS regulations and to assessing their vulnerability to exploitation and their expected response to recovery plans. Despite the socio-economic importance of dusky grouper, growth studies are few and limited in their results. The high unit value and relative scarcity of *E. marginatus* are important obstacles to research, in particular for obtaining samples of the larger, older specimens that are unusual in the catches. Although it is widely accepted that

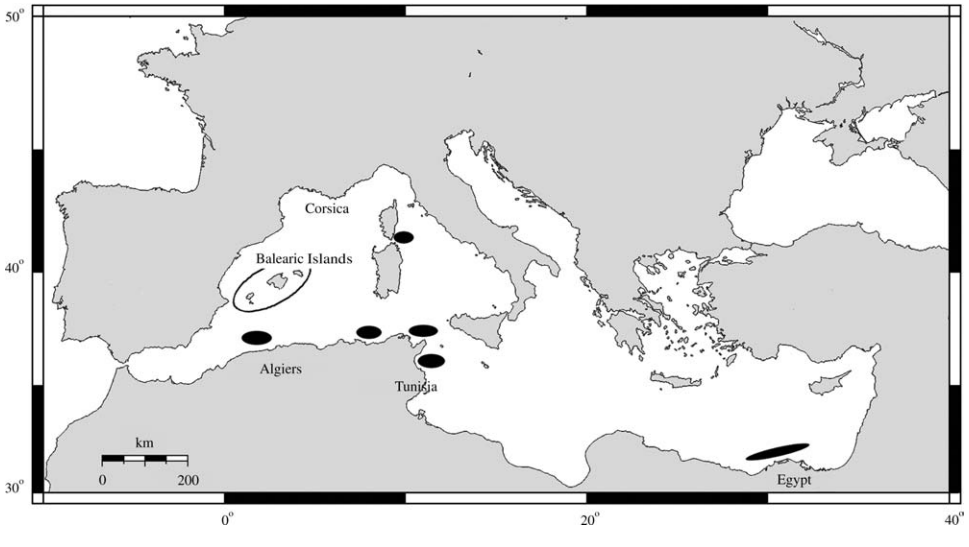

FIG. 1. Location of the Balearic Islands (study area) and of previous growth studies of *Epinephelus marginatus* carried out in the Mediterranean Sea (⬬).

E. marginatus is a long-lived species, controversy exists in respect to its life span and its relationship with fishing pressure.

Most studies of the age and growth of *E. marginatus* refer to southern Mediterranean exploited populations where the species is most abundant (Bruslé, 1985). Studies were made with specimens collected by spear fishing in shallow waters off Tunisia (Bouain, 1986; Chauvet, 1988) and Algeria (Chalabi *et al.*, 1992; Kara & Derbal, 1995), by commercial fishing at Lavezzi Island Marine Reserve (Corsica) (Bouchereau *et al.*, 1999) and by experimental longline fishing of a virgin population off Egypt (Rafail *et al.*, 1969). Finally, Azevedo *et al.* (1995) studied the growth of juveniles captured in tide-pools off Portugal. With the exception of Chauvet (1988), who used whole otoliths and scales, all authors employed scales for ageing, lengths at age were backcalculated, and the annual periodicity of ring formation was not validated due to the lack of seasonality in the samples or to the low number of specimens examined.

This study examined for the first time the growth pattern of *E. marginatus* based on age readings from sectioned otoliths. The study was in three parts: 1) analysis of the structure and growth pattern of the otoliths, including the assessment the periodicity of band formation and of the relationship between otolith size and fish size; 2) estimation of ages from whole and sectioned otoliths and comparison of the performance of the two ageing methods; and 3) estimation of mean L_T at age and growth parameters of the species and discussion of possible causes of variability in growth and longevity in the Mediterranean Sea.

MATERIALS AND METHODS

The specimens of *E. marginatus* came from three sources: 1) spear-fishing championships carried out from 1999 to 2003 ($n = 187$); 2) spear-fished dusky grouper smaller than the MLS ($n = 94$) caught for the purpose of this study (special fishing permit given by the local fishing authorities); and 3) artisanal bottom longline and trammel net commercial fishery catches ($n = 77$). These fishing methods exploit different depth segments of the population although their limits overlap: the recreational spear fishery occurs at 0 to 40 m depth (Coll *et al.*, 2004), while the artisanal fishery targets dusky grouper from 20 to 65 m depth (S. Mallol & R. Goñi, pers. comm.).

For each specimen L_T was measured to the nearest mm and mass (M) to the nearest g. Sex was determined histologically in all specimens except in a few from the spear fishing championships where this was not possible and in specimens <19 cm L_T. Sagittal otoliths were removed from each fish, cleaned and stored dry or in distilled water.

OTOLITH STRUCTURE AND GROWTH PATTERN

The otolith terminology of Secor *et al.* (1995) was used throughout this study. The terms anterior, posterior, dorsal, ventral, proximal and distal face refer to the position of the otolith relative to its original orientation in the fish (Fig. 2).

To study the growth pattern of the otolith the following measurements were made in a sample of 210 whole otoliths [Fig. 2(a)]: length [L_O: distance from the anterior (rostrum) to the posterior end taking into account its curvature], breadth (B_O: dorso-ventral distance at widest point), thickness (T_O: distal–proximal maximum distance) and mass (M_O) measured as dry mass (precision of 0·001 g) in a sub-sample of 103 otoliths. The relationship between the various otolith dimensions and fish L_T was examined by regression analysis.

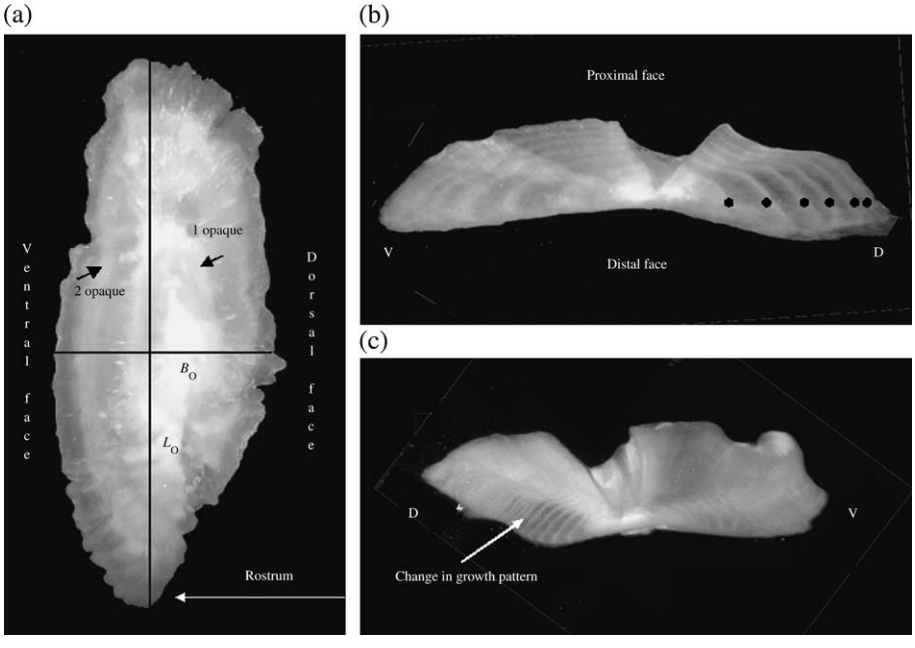

FIG. 2. (a) Distal face of a whole sagittal otolith (×20) from a 2 year-old *Epinephelus marginatus* female caught in March. The axes along which the otolith length (L_O) and breadth (B_O) measurements were made are indicated, together with the ventral and dorsal face, the rostrum and the first two opaque bands. (b) Transverse section of a right sagittal otolith (×20) from a 45 cm total length (L_T) female caught in October and estimated to be 6 years-old, the opaque increments are marked (●). (c) Transverse section (×10) of a left sagittal otolith from a 100·2 cm L_T male caught in August and estimated to be 42 years old and showing the transition from broad to narrow increments. Dorsal (D) and ventral (V) faces of the otolith are indicated in both transverse sections.

AGE ESTIMATION, BIAS AND REPRODUCIBILITY

Otolith preparation

As observations of whole otoliths showed the clearest band pattern in the dorso-ventral plane, otoliths were sectioned transversally (Fig. 2). Before sectioning whole otoliths were browned at 200° C for 5 min to increase band contrast. Following the technique described by Piñeiro *et al.* (1996), otoliths were embedded in dark polyester resin, cut in 1·4 mm thick sections and sections were mounted on glass slides. When both sagittae were available the right one was sectioned. Whole otoliths were immersed in glycerine for 2 to 72 h to improve band contrast.

Age reading

Otoliths (whole and sectioned, when available) were examined by two readers at low magnification (×6 to ×20) using a stereomicroscope with reflected light against a black background. Under these light conditions translucent bands appear dark and opaque ones appear white. Counts of the opaque bands, preferably on the dorsal face, were used to estimate ages except in otolith sections of older specimens, where opaque bands were most clearly defined and easily counted across the proximal face on either side of the acoustic sulcus [Fig. 2(b)].

The identification and time of formation of the first opaque band was assessed in 16 young-of-the-year (YOY) individuals, ranging in size from 6·6 to 15·3 cm L_T, collected

from November 2002 to August 2003. YOY dusky grouper are first observed in shallow waters at the beginning of October (unpubl. data). The annual periodicity and seasonality of band formation was assessed by examining the monthly evolution of the proportion of opaque and translucent edges in otoliths with up to eight opaque bands. The shrinkage of the width of the band near the edge and the distortion of the otolith edge, make the recognition of the marginal band very doubtful in older specimens. Once the first annual band was identified and the annual periodicity of band formation and their seasonality validated, each specimen was assigned to a year class, taking into account the date of capture, the edge type (opaque, translucent) and the number of opaque bands counted. Systematic differences in estimated age (bias) between readers were assessed by age bias plot and the reproducibility of the age interpretation (precision) was determined by the coefficient of variation (CV) (Campana *et al.*, 1995).

Age estimates from whole and sectioned otoliths

A sample of 204 specimens from 6·8 to 105·6 mm L_T were used to compare age estimates from whole and sectioned otoliths by plotting the age difference between both readings against the reading from the sectioned otoliths.

GROWTH

Total length and mass relationship

The L_T (cm) and M (g) relationship was calculated according to the equation $M = aL_T^b$ where a and b are regression coefficients. Deviation of the allometric coefficient b from the theoretical value of isometric growth ($b = 3$) was tested by t-test (Underwood, 1997).

von Bertalanffy growth parameters

Total length-at-age data were fitted to the von Bertalanffy growth model by using the non-linear curve-fitting routines of SYSTAT, with least-squares estimation as the loss function. In the model, age was expressed as a decimal number where the decimal part represents the proportion of the year from date of capture to the birthday of the species. Dusky grouper is a multiple spawner, and in the study area, the spawning season extends from June to September, peaking in July to August (Reñones *et al.*, 2004). Thus, 1 July was used as the birth date for age calculations.

RESULTS

Epinephelus marginatus specimens ranged from 6·6 to 105·6 cm L_T ($M = 4$–$22\,290$ g) with a modal size of 45–50 cm L_T (Fig. 3). Due to the seasonality of the fishery *c.* 80% of the specimens were caught between June and December.

OTOLITH STRUCTURE AND GROWTH PATTERN

The size of whole otoliths increased with increasing fish size but the rate of otolith growth relative to L_T was not constant along all axes and the variability increased with fish size. The L_O was the only otolith dimension linearly related to L_T ($L_O = 3{\cdot}48 + 2{\cdot}24L_T$, $r^2 = 0{\cdot}96$). In contrast, the rate of growth of B_O declined as L_T increased ($B_O = 0{\cdot}41L_T^{0{\cdot}71}$, $r^2 = 0{\cdot}98$). A piecewise regression fitted to the data indicated that the transition from fast to slow B_O growth took place at *c.* 45 cm fish L_T. The relationship between T_O and L_T showed an opposite trend and an exponential model provided the best fit

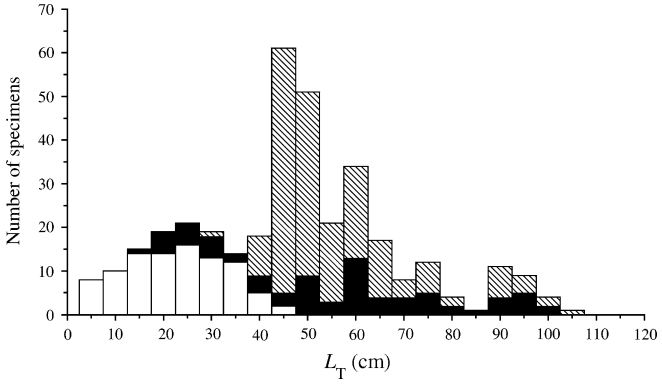

FIG. 3. *Epinephelus marginatus* total length (L_T) frequency distribution by capture method. The L_T is grouped into 5 cm intervals. □, Specimens under the minimum landing size caught experimentally with spear; ▨, specimens caught in spear fishing championships; ■, specimens caught with longline or trammel nets by the artisanal fishery.

($T_O = 0{\cdot}84L_T^{0{\cdot}02}, r^2 = 0{\cdot}93$). The T_O increased with L_T at a fixed rate up to a size of 67 cm, and at a greater rate afterwards. Finally, M_O increased exponentially with L_T ($M_O = 0{\cdot}00002L_T^{2{\cdot}26}, r^2 = 0{\cdot}98$); despite the high proportion of the variance explained by the model, the otoliths of largest individuals were in most cases heavier than predicted. This was attributed to the lack of fit at the large end of the range because of the predominance of small specimens in the sample (73% <50 cm L_T).

Dusky grouper otoliths showed the typical pattern of teleosts, without the presence of accessory growth centres. Otoliths had an alternating sequence of narrow opaque and broad translucent bands that became progressively narrower and of similar width as the number of bands increased (Fig. 2). Two different patterns were observed in otoliths with seven or more pairs of bands. Bandwidth declined gradually in 39% of the otoliths, while 61% of them presented an abrupt transition from broad to narrow bands. The transition occurred between the fourth and ninth (more frequently the fifth) pair of bands [Fig. 2(c)].

AGE ESTIMATION, BIAS AND REPRODUCIBILITY

Otoliths of YOY specimens caught from November to March (6·6 to 12·3 L_T) did not present any opaque band, whilst otoliths of specimens caught from April to August (size >8·4 mm L_T) presented a distinct opaque edge which was the first annual band or annulus (Fig. 2). The radius of the first annulus, measured in a sample of 100 otoliths from fish of the whole size range studied, ranged from 0·54 to 1·08 mm (mode 0·7 mm).

Opaque margins were observed from the beginning of March to mid-August, with a peak from April to June (Fig. 4). Although few specimens were available to estimate the proportion of opaque margins during February to May, the observed values, together with those from later months indicate that, in specimens up to 8 years old, only one opaque band is formed every year during

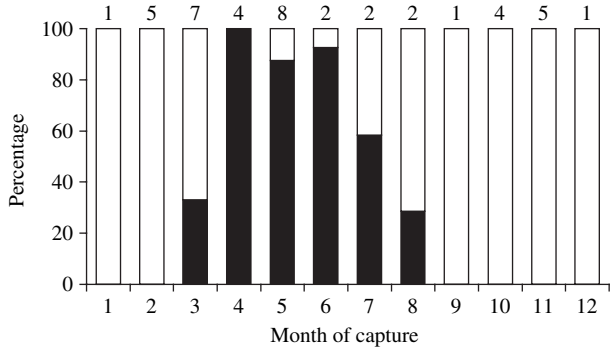

FIG. 4. Monthly percentage of opaque (■) and translucent (□) edges in otoliths of *Epinephelus marginatus*. The number of specimens examined per month is indicated above each bar.

spring to summer. Although it has not been possible to demonstrate in older grouper that only one annulus is formed every year, it was assumed that this pattern of otolith growth was maintained through the life of the fish.

The CV of age estimates was 6·3% for sectioned and 10·0% for whole otoliths. After age estimates were adjusted to allow for discrepancies between readers, which were mainly attributable to the identification of the fist opaque band, and in older specimens to the interpretation of the otolith margin, the CV was reduced to 4·2 and 8·4% in sectioned and whole otoliths respectively, with no systematic differences between readers. Although there is no *a priori* value of CV for ageing studies, a value of 5% has been indicated as a quality reference point for readings in otoliths of fishes of moderate longevity and reading complexity (Campana, 2001).

In whole otoliths kept dry or observed only a few days after extraction, the internal macrostructure was easily discerned. The estimated age from whole otoliths ranged from 0 to 52 years (for a 94·0 cm L_T specimen). Age could not be confidently estimated in 15·2% of the otoliths. The otoliths of specimens $<$40 cm L_T did not present interpretation problems, but above that size the proportion of unreadable whole otoliths increased up to 56% in specimens $>$80 cm L_T (Table I). The number of annuli observed in the sample of sectioned otoliths used to compare the performance of the two age reading methods ranged from 0 in the smaller fish to 60 in a fish of 94·0 cm L_T. Overall, 4·4% of sectioned otoliths could not be interpreted but, in contrast with whole otoliths, the percentage of unreadable otoliths decreased with fish size (Table I). This was in part due to problems with the sectioning of small otoliths.

There was an increasing divergence in annuli counts from whole and sectioned otoliths as the number of annuli grew. In general, the age estimated from whole otoliths was lower than that estimated from sections; this tendency increased after 10 annuli (Fig. 5). The mean age estimated from the sections corresponding to discarded whole otoliths from fish $>$60 cm L_T was significantly greater than the mean age from readable whole otoliths from fish of the same size range (t-test, d.f. $= 29$, $P < 0{\cdot}01$). The difference was not significant in the size classes $<$60 cm L_T.

TABLE I. Number of otoliths examined (n) for comparing the performance of ageing methods and the percentage of whole and sectioned otoliths of *Epinephelus marginatus* for which age could not be estimated (per cent unreadable) per 20 cm total length class

	L_T class (cm)					
	<20	20–40	41–60	61–80	>80	Total
n	19	36	84	47	18	204
Per cent unreadable whole otoliths	0	0	11·9	23·4	55·6	15·2
Per cent unreadable sectioned otoliths	26·3	5·6	1·2	2·1	0	4·4

GROWTH

Total length and mass relationship

The L_T (cm) and M (g) relationship of dusky grouper in the study area was $M = 0{\cdot}0098L_T^{3{\cdot}14}$ ($n = 358$, $r^2 = 0{\cdot}99$, S.E. of $b = 0{\cdot}01$). The allometric coefficient differed significantly from 3 (t-test, d.f. = 356, $P < 0{\cdot}001$), revealing a greater than expected increase in M relative to L_T during growth.

Growth parameters

Since no differences in the age estimates from whole and sectioned otoliths were apparent for the first ages, and in order to include as many observations as possible in the estimation of mean L_T at age, age estimates from whole otoliths of small fish whose sections could not be read were also used.

Age estimates of 349 specimens (97% of the samples) ranged from 0 to 61 years. The sample was dominated by specimens aged 1 to 8 years (mode 6 years) and 29 fish were estimated to be >30 years-old (Fig. 6). Females' age ranged from 2 to 52 years (19·5 to 100·3 cm L_T), transitionals' from 8 to 17 years (53·0 to 76·9 cm L_T) and males' from 7 to 61 years (58·4 to 105·6 cm L_T).

The growth in L_T of the dusky grouper was well described by the von Bertalanffy growth equation (Table II). The L_T at age increased rapidly during

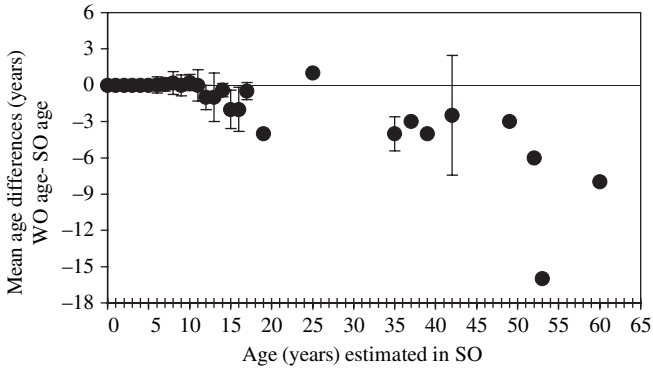

FIG. 5. Mean ± S.D. difference between ages estimated in whole (WO) and sectioned (SO) otoliths of *Epinephelus marginatus* plotted against the age estimated in sectioned otoliths.

the first 5 years (Table III and Fig. 6). Beyond this age, the growth rate declined rapidly and almost ceases after age 30 years. Variability of L_T at age increased after the period of fast growth causing the overlapping of successive age-classes (Table III). In general, spear-fished specimens showed larger L_T at age than predicted by the model, while specimens from the artisanal fishery were on average smaller than predicted (Fig. 6).

DISCUSSION

OTOLITH STRUCTURE AND GROWTH PATTERN

Sagittal otoliths of *E. marginatus* fulfil the three criteria required to be useful for ageing purposes: 1) the otoliths must grow through the life of the fish; 2) they must display an interpretable pattern of bands; and 3) the bands must be formed in a regular and determinate time scale (Fowler, 1990). *Epinephelus marginatus* otoliths grow continuously with L_T, but growth becomes asymmetric over time. Initially, otoliths grow symmetrically along the three dimensions, but while otolith length grows at a constant rate as a function of fish size, the growth rate along the dorso-ventral axis declines after a few years of life, coinciding with sexual maturity. A second change occurs later with an increased rate of growth in the proximal–distal edge. Similar asymmetries in otolith growth described in other fish species occur because the deposition of new material takes place predominantly in the proximal face of the otoliths, mainly along the sulcus region, thickening the structure (Beamish & McFarlane, 1987; Casselman, 1990). This allometric pattern of otolith growth agrees with the observation made when reading sectioned otoliths, whose bands are more easily discernible in the proximal face as age increases, and is the probable cause of the age discrepancies between whole and sectioned otoliths.

Dusky grouper up to 8 years-old form a single annulus each year during spring and summer. The first annulus was observed during the first spring when fish are 6 to 9 months-old. The variability in the width of the first annulus may

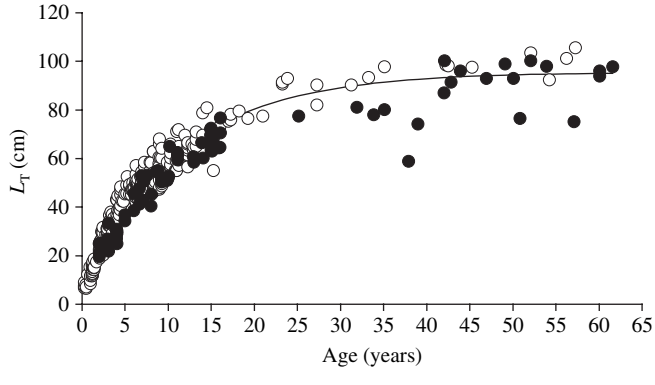

FIG. 6. Total lengths at age of *Epinephelus marginatus* and fitted von Bertalanffy growth curve (see Table II) for specimens caught by spear fishing in shallow water (○) and those caught in the artisanal fishery in deep water (●).

TABLE II. Parameter estimates ± S.E. of the von Bertalanffy growth equation of *Epinephelus marginatus*

Parameter	Estimate ± S.E.
L_∞	95·6 ± 1·1
K	0·087 ± 0·003
t_0	−1·12 ± 0·13

reflect differences in hatching time due to the protracted spawning period of the species in the area. In previous studies of age and growth of *E. marginatus* no validation of the periodicity of band formation in scales or otoliths was done due to the lack of seasonality in the samples, and it was assumed that the opaque band is formed in May (Chauvet, 1988) or June (Bouain, 1986) in response to the intensification of the trophic activity of the species. Present results confirm that opaque bands start to be laid down in spring, coinciding with the seasonal increase in water temperatures (Fernández de Puelles *et al.*, 2003) and the beginning of gonad maturation (Reñones *et al.*, 2004). The fact that it develops at the same time in juveniles and adults suggest that it is not directly related to somatic growth or to reproductive activity. The mechanisms that regulate the pattern of otolith band formation are not well understood, but the most accepted hypothesis is that band development is an independent physiological process directly responsive to environmental variations (Wright *et al.*, 2002).

AGE ESTIMATION, BIAS AND REPRODUCIBILITY

Whole and sectioned otoliths are useful for ageing specimens up to 10 years-old, but above this age whole otoliths yield lower age estimates than sections and the otoliths of the slow growing and oldest fish are selectively excluded from the sample as unreadable. Consequently, if whole otoliths are used to estimate ages, *E. marginatus* longevity is underestimated and the growth rate is overestimated. Studies assessing the performance of whole and sectioned otoliths as ageing structures have yielded diverse results but, most suggest that whole otoliths underestimate the age of older fish; the magnitude of the age difference and the age at which the divergence occurs is species-specific (McFarlane & Beamish, 1990; Ferreira & Russ, 1994; Smith *et al.*, 1995; Rocha Olivares, 1998; Morales-Nín *et al.*, 2005).

GROWTH PATTERN

Epinephelus marginatus is a long-lived species that, after a short period of fast growth, grows slowly during most of its life. The maximum estimated age of 61 years found in this study considerably extends previous estimates (Table IV). Greater longevity is a general result of the use of sectioned otoliths to estimate age in a large number of species (Beamish & McFarlane, 1987). Chauvet (1988), in the only study based in whole otoliths and scales, reports a maximum age of 36 years. All the other studies used scales as the ageing structure (Table IV) and in those studying a similar size range, the oldest fish reported was 19

TABLE III. Number of specimens and mean ± S.D. and range of total length (L_T) and mass (M) at age of *Epinephelus marginatus*

Age (years)	n	Mean ± S.D. L_T (cm)	L_T range (cm)	Mean ± S.D. M (g)	M range (g)
0	10	9·1 ± 3·1	6·6–15·3	13 ± 14	4–43
1	21	14·4 ± 2·6	8·4–18·6	47 ± 24	8–107
2	35	25·3 ± 3·3	19·0–31·6	266 ± 116	106–590
3	20	33·2 ± 2·6	29·0–38·0	617 ± 166	383–940
4	26	40·3 ± 4·4	33·1–48·3	1141 ± 420	582–2020
5	15	47·7 ± 2·2	45·0–52·6	2017 ± 288	1646–2368
6	42	48·9 ± 3·2	42·1–57·1	2029 ± 418	1234–2878
7	28	51·2 ± 3·6	45·6–58·5	2283 ± 512	1642–3720
8	28	53·3 ± 4·8	45·2–62·8	2573 ± 769	1549–4333
9	17	55·4 ± 6·5	47·3–68·0	3013 ± 1246	1750–5854
10	13	58·7 ± 4·8	52·0–66·0	3439 ± 884	2043–4969
11	15	63·0 ± 4·8	55·1–71·8	4582 ± 1521	3034–8690
12	10	63·5 ± 3·1	56·5–66·5	4479 ± 765	3109–5350
13	8	63·9 ± 6·5	50·5–71·0	4585 ± 1362	2030–6371
14	7	71·1 ± 7·1	60·2–80·8	7106 ± 2250	3900–10 250
15	9	64·7 ± 4·7	55·0–72·5	4814 ± 1102	2724–6360
16	4	71·2 ± 5·1	64·6–76·6	6098 ± 1514	4400–7980
17	3	76·5 ± 1·6	75·4–78·3	7407 ± 1135	6450–8662
18	1	79·5		9086	
19	1	76·5		8175	
21	1	77·5		9490	
23	3	91·7 ± 1·2	90·7–93·0	15 325 ± 1452	13 744–16 600
25	1	77·4		9000	
26	2	86·2 ± 5·9	82·0–90·3	11 812 ± 2542	10 014–13 610
31	2	85·7 ± 6·6	81·0–90·3	11 302 ± 3185	9050–13 555
33	2	85·7 ± 10·8	78·0–93·3	11 710 ± 4680	8400–15 019
36	2	89·0 ± 12·5	80·2–97·8	12 315 ± 5211	8630–16 000
37	1	58·8		3400	
39	1	74·1		7950	
42	5	95·1 ± 5·6	87·0–100·2	15 302 ± 2893	12 000–17 808
43	1	96·0		13 600	
45	1	97·5		17 246	
46	1	93·0		15 700	
49	1	99·0		17 000	
50	2	84·8 ± 11·7	76·5–93·0	13 400 ± 1979	12 000–14 800
52	2	101·9 ± 2·3	100·3–103·5	19 230 ± 806	18 660–19 800
53	1	98·0		16 300	
54	1	92·4		14 569	
56	1	101·2		19 386	
57	2	90·4 ± 21·6	75·1–105·6	14 730 ± 10 691	7170–22 290
60	2	95·0 ± 1·4	94·0–96·0	15 050 ± 1060	14 300–15 800
61	1	97·8		16 000	

years old (100 cm L_T) (Bouchereau *et al.*, 1999). Comparative studies of scales and otoliths (whole or sectioned) as ageing structures in other species report that scales of old fishes may underestimate age relative to otoliths (Beamish &

TABLE IV. Study area, ageing structure (Sc, scales; Wo, whole otoliths; So, sectioned otoliths), capture method, number of specimens sampled (n) total length, age range and von Bertalanffy growth parameters of *Epinephelus marginatus* previously reported from the Mediterranean Sea. Original data in standard length (L_S, cm) was transformed to L_T (cm) by the equation: $L_T = 0{\cdot}84 + 1{\cdot}146\,L_S$ ($n = 222$, $r^2 = 0{\cdot}99$) (unpubl. data)

Area	Ageing structure	Capture method	n	L_T (cm)	Age range (years)	L_∞	K	t_0	Reference
Egypt	Sc	Experimental fishing	251	16·6–47·5	1–7	80·0	0·112	−1·09	Rafail *et al.* (1969)
East Tunisia	Sc	No data	97	18·0–103·0	1–16	213·0	0·028	−1·53	Bouain (1986)
North Tunisia	Sc and Wo	Spear fishing	270	5·3–118·0	0–36	114·5	0·093	−0·75	Chauvet (1988)
East Algeria	Sc	Spear fishing	34	19·7–56·7	1–7	78·5	0·160	−0·73	Kara & Derbal (1995)
West Algeria	Sc	Spear fishing	39	26·9–86·8	1–13	177·4	0·073	−1·24	Chalabi *et al.* (1992)
Lavezzi Islands	Sc	Commercial fishing	24	20·0–120·0	1–19	135·9	0·079	−0·80	Bouchereau *et al.* (1999)
Balearic Islands	So	Spear and commercial fishing	349	6·6–105·6	0–61	95·6	0·087	−1·12	Present study

McFarlane, 1987; Rocha Olivares, 1998). This is caused by the different growth pattern of both structures in relation to somatic growth, which influence their ability to reflect annual growth as age increases (Beamish & McFarlane, 1987; Casselman, 1990).

In addition to the ageing methods, other factors may affect the estimated longevity of fishes. The exploitation history of fish populations affects their demography and sustained heavy exploitation results in truncated age structures by removing the largest (and presumably older) individuals (Ricker, 1969; Goñi, 1998). Here this explanation does not seem plausible because, although the exploitation history of the *E. marginatus* populations studied are not known, the long tradition of dusky grouper fishing in the Balearic Islands (Coll *et al.*, 2004), particularly in shallow waters, suggests that exploitation rates are not less than they were 7 to 30 years ago in the other Mediterranean populations studied. In fact, greater fishing pressure in the Balearic Islands is suggested by the presence of transitionals and males of relatively small size (and low age), close to the females' size at maturity, and smaller than reported for other Mediterranean populations (Bruslé, 1985; Chauvet, 1988; Kara & Derbal, 1999; Marino *et al.*, 2001). Subject to high fishing pressure, the species in the Balearic Islands could have lowered the size and age of sex change in order to maintain the reproductive capacity, as has been indicated for other hermaphrodite species (Sanchez Lizaso *et al.*, 2000). Alternatively, environmental conditions and availability and type of food resources could affect fish growth rates and thus lengths at age (Wootton, 1990), but not longevity. Nevertheless, any attempt to understand the effects of fishing or of the environment on demography is confounded by the problems associated with the ageing method (Beamish & McFarlane, 1995).

The hypothesis that this species could attain older ages than observed was already proposed by Chauvet (1988), on the basis of one specimen kept in captivity for >50 years reported by Miraglia (1935). Results of monitoring studies of *E. marginatus* for >15 years in western Mediterranean marine protected areas (Garcia-Rubies *et al.*, 2005) and knowledge of its biology (Harmelin & Harmelin-Vivien, 1999) also lend support to the great longevity of the species reported here. This species along with *Epinephelus caninus* (Valenciennes, 1843) (55 years-old; Morales-Nín *et al.*, 2005) and *Epinephelus nigritus* (Holbrook, 1855) (41 years-old; Manooch & Manson, 1987) are therefore the oldest *Epinephelus* species aged to date.

Epinephelus marginatus reaches almost 50% of the estimated L_∞ (*i.e.* 47·7 cm L_T) and 2 kg during the first 5 years of life. Beyond this age, the growth rate slows down to <3 cm year^{-1} up to *c.* age 30 years, when growth in L_T almost ceases. This change in growth pattern has been observed in both northern and southern Mediterranean populations. The physiological event proposed to be responsible for this sudden reduction in somatic growth is the onset of maturity (Chauvet, 1988; Kara & Derbal, 1999). In the Mediterranean Sea, the reported size at 50% maturity for females ranges from 43·8 cm standard length (51 cm L_T) in the Sicilian Channel (Marino *et al.*, 2001) to 57 cm L_T in Algerian waters (Kara & Derbal, 1999). In the Balearic Islands, the smallest mature female observed measured 38·6 cm L_T and the estimated size at 50% maturity is 49 cm L_T (O. Reñones, A. Grau, X. Mas & F. Riera, unpubl. data). These sizes are coincident with the L_T where the reduction of somatic growth was observed in this study.

The L_∞ of 95·5 mm L_T estimated in this study and the largest individual sampled (105·6 cm L_T) are considerably lower than the maximum of 120 cm L_T given for the species (Heemstra & Randall, 1993). This is most likely due to the absence or rarity of the large and faster growing individuals in the highly exploited Balearic Islands population, particularly in the case of the shallow, fast growing component of the population.

Epinephelus marginatus exhibits high individual variation of L_T at age. This makes L_T a poor predictor of age for this species, especially after age 5 years when growth slows down. Several factors, as mentioned above, may be put forward to explain some of this variability. Sea-surface temperature in the study area varies from 13·5° C in February and March to 28° C in August and September, and seasonal stratification within the first 50 m of the water column creates sharp temperature gradients of up to 12° C (Fernández de Puelles *et al.*, 2003). The spawning season of *E. marginatus* takes place in summer extending for *c.* 3 months.

Thus, early and late spawning coincide with the rise and fall of water temperature respectively (Fernández de Puelles *et al.*, 2003). Hence, differences in hatching time may produce intra- and interannual differences in growth during the first years of life. Also, *E. marginatus* shows a wide depth distribution. Two year-old dusky grouper in this study were caught from the coast down to 40 m depth. Older fish were caught by spear-fishing, from near the coast or by the artisanal fishery, down to 65 m depth. Although the species shows a positive depth–size relationship, individuals within each age group exhibit a wide depth distribution that seems more related to the availability of suitable habitats than to depth itself, as observed in protected populations where the species has recolonized shallow waters (Harmelin & Harmelin-Vivien, 1999; Reñones *et al.*, 1999; La Mesa *et al.*, 2002; Bodilis *et al.*, 2003). Total length at age of shallow water, spear-fished grouper were on average larger than mean L_T at age of deeper water dusky grouper caught by the artisanal fishery. The higher seawater temperature and food availability in shallow waters, both favouring fish growth (Wootton, 1990), could produce these differences.

Mean L_T at age during the period of fast growth observed in this study are similar to those reported for the species in Tunisia (Chauvet, 1988), Algeria (Kara & Derbal, 1995) and Lavezzi Islands (Bouchereau *et al.*, 1999), but afterwards L_T at age are lower. This discrepancy and the greater longevity reported here, result in a slower increase in L_T with age and, as a consequence, a greater discrepancy between this and previous growth curves (Tables II and IV). As shown earlier, this divergence may be largely attributed to the ageing structure used, although other factors such as the degree of exploitation or environmental conditions cannot be discounted.

In summary, the results of this study show that ageing whole otoliths of *E. marginatus* leads to overestimating mean L_T at age and growth rates and to underestimating maximum ages. It is clear that dusky grouper is a long-lived species which approaches its maximum size very gradually. The implications of slow growth are numerous. The most prominent in the context of an overfished species is that population turnover is lower than expected and its response to recovery plans slower than predicted with the knowledge available until present. Longevity, in turn, is a bet-hedging strategy for the persistence of a population

(Secor, 2000), and may explain the ubiquity of *E. marginatus* despite its overfished status. Due to its slow growth, late maturation, hermaphroditism and low natural mortality dusky grouper should be considered 'conservation dependent'. Thus, fishery regulations of *E. marginatus* should aim not only to prevent growth overfishing through minimum landing sizes, but also to prevent recruitment overfishing through the protection of the oldest population components and of those occupying shallow water, where the most suitable conditions for growth are found.

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